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Star volumes of villi and intervillous pores in placentae from low and high altitude pregnancies

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ABSTRACT

Histological sections of placentae from pregnancies completed at low altitude (400 m) and high altitude (3600 m) in Bolivia were analysed using a stereological estimator of the star volumes of villous 'domains' and intervillous 'pores'. The purpose was to test whether or not differences in the overall volumes of these compartments are accompanied by changes in their geometrical relationships. Whilst total placental volume did not vary with altitude, the total volume of villi declined by about 25% and total intervillous volume increased by 40% at high altitude. The star volume of villi also decreased by 25% (from 1.5×10^6 μm^3 at low altitude to 1.1×10^6 μm^3 at high altitude) whilst the star volume of intervillous pores increased 4-fold (from 87×10^6 μm^3 to 461×10^6 μm^3). These figures imply that villous domains decrease in size but may be constant in number. The most likely explanation is that villous trees at high altitude are scaled-down versions of their low-altitude counterparts. By contrast, although the intervillous pores enlarge they may decrease in number in the highland organ. This may reflect a change in the number of maternal cotyledons or in the spatial arrangement of villous trees.

Key words: Placenta; villi; intervillous space; star volume.

INTRODUCTION

The human placenta has been envisaged as a porous medium (Schmid-Schönbein, 1988) in which porosity is determined by the spatiotemporal interplay between villous arborisations and the maternal intervillous space (IVS). The villi can be viewed as a network of connected domains (formed by branches and bridges) and the IVS as a set of confluent pores whose number, shapes and sizes are partly determined by the villous trees. Their geometric properties and relationships influence haemodynamics and vascular and diffusive conductances.

Recently, Kosanke et al. (1993) described an approach for studying the topological properties of villous arborisations. While providing extremely useful information about villous branching patterns, this approach requires extensive serial sectioning and does not yield information on consequences for the geometry of the IVS. More recently, we applied an alternative but complementary approach suitable for use with random sections and based on the 3-dimensional (3D) quantity known as 'star volume' (Gundersen & Jensen, 1985; Gundersen, 1986; Vesterby et al. 1988, 1989; Vesterby, 1993). This quantity was used to monitor the changing geometric relations between intervillous villi and the IVS during gestation (Mayhew & Wadrop, 1994). We found that the star volumes of IVS pores and villous domains declined and that these alterations were compatible with known mechanisms of villous proliferation and maturation (Mayhew & Wadrop, 1994). Applications to human placentae have shown that, provided processing artefacts are corrected, there is excellent agreement between values of IVS star volume obtained using different fixation and embedding procedures (Karimu & Burton, 1993; Mayhew & Wadrop, 1994).

Adaptations of placental morphology also occur during high-altitude pregnancy (Jackson et al. 1985, 1987*a*, *b*, 1988*a*, *b*; Mayhew et al. 1990). The highland placenta has a more voluminous IVS but an impoverished villous growth as expressed in terms of the total volume, surface area and length of villi (Jackson et al. 1987*a*, *b*). Whilst it is reasonable to expect that

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such changes in the global dimensions of villi and IVS might also affect their individual and mutual geometries, this possibility has not been investigated quantitatively. Part of the reason is the architectural complexity of the intervillous pore spaces and the inability to model them in a realistic manner. For these reasons, we have examined low and high altitude placentae for evidence of differences in the star volumes of these spaces in an attempt to understand better the physical and functional consequences of changes in the dimensions of villi and the IVS. The advantage of using star volume stems from the fact that it allows us to define a volume for spaces of arbitrary shape and size (Gundersen & Jensen, 1985; Gundersen, 1986).

MATERIALS AND METHODS

The placental samples

Details have been given previously (Haas, 1980; Haas et al. 1980; Jackson et al. 1987*a*, *b*, 1988*a*, *b*). Briefly, healthy women at no apparent obstetric risk were born, brought up and completed singleton pregnancies at 400 m (low altitude, LA) or 3600 m (high altitude, HA) in Bolivia. In addition to altitude of residence, the subjects were classified according to ethnic background and sex of newborn and showed altitudinal, ethnic and sex differences in birth (but not placental) weight. Birthweight was greater at LA vs HA, in native (Amerindian) vs non-native groups and (at least at LA) in male vs female newborn. For the present study, we analysed 69 placentae from pregnancies which, apart from 4 cases of caesarean section, ended in spontaneous vaginal delivery at term. The LA organs (n = 24) comprised 10 Amerindians (4 males, 6 females) and 14 non-Indians (7 males, 7 females). The HA organs (n = 45) comprised 16 Amerindians (7 males, 9 females) and 29 non-Indians (11 males, 18 females).

Umbilical cords were clamped within 1 min of delivery and placentae refrigerated prior to further sampling. Membranes were trimmed, the cord was cut about 5 mm from its placental insertion, and organs were weighed. Full-depth tissue pieces were immersion-fixed in isotonic formol saline and embedded haphazardly in paraffin wax. Randomly chosen blocks were cut at section thicknesses of 3–5 μm, mounted on glass microslides and stained by the Masson trichrome method (Bancroft & Cook, 1984).

To estimate IVS star volumes, microslide images were projected onto the workbench (final linear magnification $M = 154$ or 250) using a modified Olympus BHS microscope. This was fitted with a 100 watt halogen light source, a projection attachment and 2 step-motors to drive the microscope stage at preselected distances in x and y directions (Bico A/S, Denmark). The step-motors allowed us to select fields of view in a systematic random fashion (Gundersen & Jensen, 1987; Mayhew & Burton, 1988; Mayhew, 1992*a*, *b*). For estimating the global and star volumes of villi, 2 sets of 20–30 systematic random microscopical fields were selected and black-and-white micrographs ($M = 250$) prepared together with colour slide transparencies (projected onto a wall at $M = 3980$). All magnifications were calibrated using micrometer scales as external standards.

Stereological estimations

Microscopic fields were analysed blind by the same person.

Global quantities. Compartment volume densities (cm^3/cm^3) were estimated by point counting methods (Jackson et al. 1987*a*, *b*). Absolute volumes (cm^3) were calculated from fresh placental weight and volume densities via specific gravity (taken to be 1.05 g/cm^3; Laga et al. 1973). Surface areas (in m^2) were calculated from surface densities estimated by counting test intersections (Jackson et al. 1987*b*). Tissue processing distortions were corrected using the diameters of red blood cells as internal calibration standards (Mayhew & Burton, 1988; Simpson et al. 1992) and assuming that shrinkage distortions were uniform and concentric. The average diameter of an erythrocyte is about 7.5 μm (see Weiss, 1983; Weibel, 1984) but in our preparations the diameter was 5.0–6.0 μm in both LA and HA organs. There were no significant altitudinal or ethnic differences. Whilst these corrections might not reproduce the true in situ situation, they allowed us to maintain internal consistency and should permit valid comparisons between groups.

Star volumes. Star volume, V(star) in μm^3, provides a direct and unbiased estimate of volume which has a strict mathematical definition, i.e. the volume of all parts of a 3D space which are visible when viewed in every direction from a given point within it. The mean value is averaged over a set of randomly sampled points. It is not easy to appreciate this value for a very complex shape but its significance can be appreciated using some simple illustrations. For a sphere, the star volume will correspond to sphere volume because the inner surface of a sphere can be seen in all directions from any point within it. The same is true for all convex objects. For more complicated objects, star

volume will be less than the total volume. For example, an internal view of the kidney from a point located in one pole would not include areas obscured by the curve of the hilum. As a gross oversimplification of the architecture of the human placenta, imagine a closed cubical room supported by cylindrical pillars running from floor to ceiling. The star volume of the room space would be less than that of the cube because views from points in certain directions would be obscured by pillars. Altering only the diameters or numbers of pillars would decrease star volume whereas introducing see-through holes in the pillars would increase it. Clearly, the packing, size and topology of villi in the placental IVS will influence its star volume in similar ways.

Estimates of placental star volumes were obtained by measuring point-sampled intercept lengths (Gundersen & Jensen, 1985) generated by projecting fields of view onto test points drawn on white cardboard. The final estimate was calculated from the mean of the cubed intercept lengths:

$$V(\text{star}) = (\pi/3) \times \overline{(l^3)}.$$

When test points fell on the IVS, we measured the lengths of randomly-oriented intercepts running without obstruction through the points and from one boundary surface (most frequently villous) to another (Mayhew & Wadrop, 1994). A similar procedure was used for villi but this time intercepts were measured through test points and between one villous surface and another. In both cases, the term 'villous surface' refers to the apical surface of the trophoblastic epithelium. The term 'boundary surface' would include nonparenchymal surfaces (e.g. intercotyledonary septa). Between 82 and 181 (average 119) intercepts per organ were measured for villi and 97–177 (average 106) intercepts for IVS. Villous star volumes were estimated for all 69 placentae whilst IVS star volumes were estimated using a subsample of 30 organs (3–4 organs per group). Observed intercept lengths were corrected for magnification and for the effects of tissue processing distortions.

Statistical analyses

Means and standard errors of means (S.E.M.s) were calculated for each group of organs. Comparisons between groups were drawn using 3-way analyses of variance (Sokal & Rohlf, 1981) for unequal sample sizes. The 3 main effects were altitude, ethnic grouping and sex of newborn infant, although first and second order interaction terms were also monitored. All data were handled and analysed using Unistat 4.50 statistical software (Unistat Ltd, London) on a Viglen 4DX266 Mini Tower system.

RESULTS

Quantitative findings are summarised in Tables 1–3.

Placental size

The volume of the placenta was not significantly different between altitudes (433 cm^3 at LA and 448 cm^3 at HA; Table 1), nor did we detect any significant ethnic, sex or interaction effects.

Global volumes and surface areas

No significant ethnic, sex or interaction effects were found for any of the estimated quantities. The volume of villi per placenta declined from 158 (10.9) cm^3 at LA to 120 (4.42) cm^3 at HA (Table 2) and this difference was significant (variance ratio, $F = 13.7$ for degrees of freedom, D.F. = 1, 61; $P < 0.001$). This was associated with a decrease in villous surface area which was 17% less at HA (5.6 compared with 6.8 m^2; $F = 7.5$; $P < 0.01$).

The IVS volume per placenta increased substantially, from 177 (11.9) cm^3 to 249 (7.86) cm^3 ($F = 25.8$; $P < 0.001$) and this matched neither the change in villous surface nor the apparent difference in placental volume. In fact, the ratio of IVS volume to villous surface increased in HA placentae from roughly 29 cm^3/m^2 to 47 cm^3/m^2 and the fractional volume of the placenta occupied by IVS rose from 41% to 56% ($F = 20.9$ and 33.9 respectively; $P < 0.001$ in both cases).

Star volumes

We detected no significant ethnic, sex or interaction effects for either of the 2 star volume estimates. HA

Table 1. *Group means (S.E.M.) for placental volumes*

	Volume (cm^3)	
Group	Low altitude	High altitude
All	433 (18.7)	448 (12.4)
Amerindian		
Male	390 (47.0)	462 (30.7)
Female	470 (38.1)	453 (16.9)
Non-Indian		
Male	432 (28.0)	468 (37.6)
Female	427 (42.0)	427 (15.4)

Table 2. *Group means (S.E.M.) for villous and intervillous volumes*

	Villous volume (cm^3)		IVS volume (cm^3)	
Group	LA	HA	LA	HA
All	158 (10.9)	120 (4.42)	177 (11.9)	249 (7.86)
Amerindian				
Male	134 (27.1)	138 (14.6)	165 (24.3)	254 (14.1)
Female	181 (24.6)	135 (9.31)	201 (40.0)	257 (14.5)
Non-Indian				
Male	160 (8.14)	117 (10.5)	156 (9.37)	251 (19.9)
Female	148 (26.3)	109 (3.93)	184 (17.0)	241 (13.2)

Table 3. *Group means (S.E.M.) for villous and intervillous star volumes*

	Villous star volume ($\mu m^3 \times 10^6$)		IVS star volume ($\mu m^3 \times 10^6$)	
Group	LA	HA	LA	HA
All	1.47 (0.130)	1.11 (0.068)	87 (53)	461 (149)
Amerindian				
Male	1.46 (0.270)	1.01 (0.153)	16 (6)	589 (526)
Female	1.18 (0.242)	1.18 (0.123)	35 (8)	540 (83)
Non-Indian				
Male	1.63 (0.264)	1.12 (0.157)	259 (187)	455 (273)
Female	1.57 (0.269)	1.11 (0.118)	19 (5)	280 (132)

placentae exhibited a significantly smaller ($F = 7.2$; $P < 0.01$) villous star volume (Table 3). The values were $1.11\ (0.07) \times 10^6\ \mu m^3$ and $1.47\ (0.13) \times 10^6\ \mu m^3$. In contrast, IVS star volume was greater at HA: $461\ (149) \times 10^6\ \mu m^3$ and $87\ (53) \times 10^6\ \mu m^3$ ($F = 4.5$ for 1, 22 D.F.; $P < 0.05$).

DISCUSSION

This study has shown that altitude-related differences in the global dimensions of human placental villi and IVS (Jackson et al. 1987*a*, *b*; 1988 *b*) are accompanied by alterations in the geometric relationships within and between these parenchymal compartments. The decrease in villous star volume in highland placentae was commensurate with the decrease in total volume of villi (roughly 25% less than in lowland controls) but the 4-fold increase in IVS star volume was far greater than the 40% increase in total volume of the maternal vascular bed.

It would be misleading to equate villous domains and IVS pores with actual units of placental architecture. Instead, they are locally defined, point-sampled regions of arbitrary space. Consequently, the mean star volume of villi depends on their dimensions (length, diameter) and topological characteristics (degree of branching, presence/absence of true intervillous syncytial bridges rather than those artefacts generated by angle of sectioning, e.g. Burton, 1986; Cantle et al. 1987; Kaufmann et al. 1987). Amongst other factors, IVS star volume depends on the global volume of the IVS (influenced at delivery by maternal blood loss following placental detachment from the uterine wall), the number, size and spatial arrangement of surrounding villous arborisations, and the episodic spurting of maternal blood into placentones from the openings of uterine arteries (Mayhew & Wadrop, 1994).

For the above reasons, it would be imprudent to try to draw firm conclusions by regarding star volumes as summative units of space. Dividing global volumes by star volumes merely gives an indication of the *theoretical* number of star volumes which could be accommodated within the total volume. However, maternal blood flow will be influenced by the real number and arrangement of IVS spaces and villi. In the case of the present findings, the theoretical number remains constant across altitudes for villous domains but is reduced at HA for IVS pores.

A reduction in villous star volume for a constant number of villous domains can be interpreted in several ways. For instance, it might signify that certain villous branches are smaller (thinner or shorter) or that all branches are smaller (i.e. each villous arbor is simply scaled down in size) or that the branching pattern is different. There is morphometric evidence that the combined length of villi is reduced at HA and this seems to affect branches in all diameter classes except that of the smallest terminal villi (Jackson et al. 1987*b*). This impoverished linear growth could produce the drop in star volume seen in the present study. However, the possibility cannot be excluded that all villous branches on all arbors are reduced in size at HA. The number of villous trees might also alter. A 25% decrease in villous volume would correspond to a roughly 10% reduction in linear dimensions if overall size altered isomorphically. Results obtained by Jackson et al. (1987*b*) suggest that villous length actually decreases by about 17% (from 36 to 30 km). This is probably within the margin of experimental error given that, at each altitude, data were pooled over ethnic groups and sexes and length frequency distributions were based on a constant diameter class interval. Finally, the question of whether or not branching pattern is affected must await topological analyses such as those undertaken by Kosanke et al. (1993).

Turning to the intervillous space, corresponding calculations indicate that the number of star volume pores theoretically containable within it is substantially reduced at HA. This might reflect the stunted growth of villi (Jackson et al. 1987*b*) or indicate a change in the number of maternal cotyledons. Kruger & Arias-Stella (1970) reported that cotyledon number in HA organs was half that of LA controls. Their proposal that this might represent a general reduction in the proportion of nonparenchymal tissues is supported by volumetric analyses (Jackson et al. 1987*a*) and is also consistent with the increase in IVS star volume seen in highland organs. A further possibility is that the packing of villous arbors is more localised at HA. If, for example, fetal cotyledons were concentrated centrally, and nonparenchymal tissue were reduced, this could leave a more voluminous blood space at the placental margin and lead to higher star volumes for the IVS. This might also contribute to the greater observed variation between organs suggested by the relatively large S.E.M. values. Further work is required to resolve these possibilities.

In terms of transplacental exchanges, villous volume and surface area are influential in determining demand and transport capacity respectively whilst the total volume of the IVS influences supply. However, other factors are clearly important. These include, for example, the packing densities and activation states of transport sites within trophoblastic and other plasma membranes, the volume of trophoblast, fetal and maternal vascular conductances and nature of the substance being transported. In the case of oxygen, the most vital of transferred molecules, transport is by passive diffusion although transport is not diffusion-limited. Oxygen consumption by the placenta itself is determined largely by the volume of metabolically-active trophoblast whilst diffusive conductance is directly proportional to villous surface area and inversely proportional to diffusion distance (Laga et al. 1973; Mayhew et al. 1986). Maternal vascular conductance depends, in part, on IVS volume. In terms of supply and demand, therefore, the HA organ seems to have adapted to provide a larger volume of maternal blood to a given villous surface (i.e. 47 compared with 29 cm^3/m^2). At least for oxygen diffusion, it is known that these changes help to maintain overall placental diffusive conductance at HA and, since birth weight is reduced at altitude, lead to a higher specific conductance when normalised for birth weight (Mayhew et al. 1990).

It is not possible to predict the likely consequences of these changed geometric relationships on placental IVS blood flow and apparent viscosity because the present morphometric data form only part of the complete picture. If blood flow in the IVS is treated as flow through a porous medium, the type of flow and attendant shear forces would be markedly different from those in a system of tubes (Dullien, 1979; Peeters & Buchan, 1989). The hydraulic diameter of a roughly homogeneous porous medium is equal to 4 times the ratio of volume to surface if they are treated as cylinders (Dullien, 1979). For the IVS pores at LA, the hydraulic diameter of $4 \times 180/70000 = 0.0103$ cm or 103 μm is obtained (see volumes and surface areas in Table 4). The corresponding value for HA organs is 167 μm. This 60% increase in diameter is equivalent to a roughly 4.3-fold increase in volume and this model-based estimate is consistent with the observed (model-independent) estimate of relative star volumes ($461/87 = 5.2$; Table 4). In order to calculate shear rates and apparent viscosities in the IVS from hydraulic diameters, firm data would be needed for the numbers of cotyledons and the total IVS blood flow in LA and HA organs and these are lacking at present.

In a study of villous star volumes in a UK series of placentae during gestation, we found that values decreased to about 1×10^6 μm^3 at term (Mayhew &

Table 4. *Summary of morphometric results obtained for the IVS and villi of different groups of placentae*

Variable	LA*	HA*	UK series†	Danish series‡
Weight of placenta (g)	455	470	475	500
Volume of IVS (ml)	177	249	184	174
Volume of villi (ml)	158	120	210	232
Surface of villi (m^2)	7	6	11	9
Length of villi (km)	36	30	81	60
Diameter of villi (μm)	75	73	58	71
V(star) villi ($\mu m^3 \times 10^6$)	1.5	1.1	0.9	dna
Theoretical number of villous domains, $\times 10^6$	105	109	233	dna
V(star) IVS ($\mu m^3 \times 10^6$)	87	461	0.4	dna
Theoretical number of IVS pores, $\times 10^6$	2.0	0.5	460	dna

* Data from present study and Jackson et al. (1987*a*, *b*); † data from Jackson et al. (1992); ‡ data from Mayhew et al. (1993); dna, data not available.

Wadrop, 1994). This value is reasonably close to present findings ($1.1–1.5 \times 10^6$ μm^3). However, previous studies on IVS star volume in human term placentae have produced values in the order of only 268 000–751 000 μm^3 (Karimu & Burton, 1993; Mayhew & Wadrop, 1994). These figures are far lower than the value of 87×10^6 μm^3 for LA organs in this Bolivian series and indicate that star volume estimates are sensitive to umbilical arterial perfusion pressures (Karimu & Burton, 1993).

It is unlikely that the IVS discrepancy can be attributed to differences in total placental weight or IVS volume. Studies on other 'lowland' term placentae conducted in our laboratories have shown that these variables are similar in Bolivian, UK and Danish series (about 475 g and 180 cm^3, respectively; see Table 4). A contributory factor could be villous growth. Total villous volume, surface area and length are less in the Bolivian organs (see also Jackson et al. 1987*a*, *b*) than in those from UK studies on gestation (Jackson et al. 1992) and Danish studies on diabetic pregnancies (Mayhew et al. 1993). Villous mean diameters are broadly similar (Table 4). It appears that villous trees are less well-developed in the Bolivian organs than in their European counterparts and the explanation for this is unknown. Whatever its cause, it is clear that the relatively greater growth of villi in European organs is consistent with denser packing of villous branches in the intervillous space and the attendant decrease in pore size and increase in pore number. However, the magnitude of the discrepancies is surprising and so other factors may be involved. One possibility is that Bolivian placentae also differ from European organs in terms of the spatial arrangement (regional packing density) of their fetal cotyledons. Systematic studies of potential ethnic differences are lacking. Finally, group comparisons will also be sensitive to confounders such as the time of cord clamping (which influences blood losses), method of fixation and perfusion pressure.

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